REMARKS

Applicants submit this Request For Continued Examination (RCE) pursuant to 37 C.F.R. §1.114 to have amendments to the specification and claims entered and considered on the merits.

Support for the amendments to the claims may be found throughout the specification, in particular, page 8, lines 16-18; page 19, line 26 to page 20, line 21; page 22, lines 19 to 28; and page 27, lines 5-9. Support for new Claims 26-28 may be found throughout the specification including page 20, line 9-21 (Claim 26); page 20, lines 9-16 (Claim 27); and page 11, line 25, to page 12, line 7 (Claim 28). No new matter is added by the amendments to the claims.

The claim amendments submitted herewith reflect subject matter as allowed in the European counterpart to the present application, EP Appln. No. 03021423.3, having a date of publication and mention of grant of January 7, 2009.

I. Specification

The Examiner has objected to the incorporation by reference of essential material, in particular the reference of the chemically modified DNA polymerase (HotStarTaq®) on page 27 of the application as disclosed in European Pat. Appln. No. 99110426, which corresponds to U.S. Pat. No. 6,183,998.

In response, Applicants have amended the specification at page 27 to delete the reference to a foreign publication and replace it with the description of hot start DNA polymerases as set forth in U.S. 6,183,998 that was intended to be incorporated. The text incorporated from U.S. 6,183,998 may be found at col. 3, lines 5-21 and 56-59, and col. 12, lines 20-21.

The amendment to the specification is a description of material previously incorporated by reference and no new matter is added by this amendment.

Entry of the amendment to the specification is respectfully requested.

II. 35 U.S.C. §103

The Examiner has maintained the rejection of Claims 1-2, 4-11, 16, 23, and 24 under 35 U.S.C. §103 as being unpatentable over Backus et al., U.S. Pat. No. 5,705,366[1], in view of Bustin, S.A., *Journal of Molecular Endocrinology*, 25: 169-193 (2000) and further in view of Birch et al., U.S. Pat. No. 5,773,258 ("Birch et al.").

[1] Applicants note that the Examiner cites U.S. Pat. No. 5,703,366 throughout the Office Action. However, U.S. Pat. No. 5,703,366 is issued to Sting et al. and is entitled "Optical Sensing With Crystal Assembly Sensing Tip".

According to the Examiner, with respect to Backus,

> "Backus discloses a method of amplification of multiple target nucleic acids in the presence of a nonionic, polymeric volume exclusion agent." (See, Office Action, page 3.)

With respect to Bustin, the Examiner states,

> "Bustin teaches an overview of the quantitation of mRNA using a variety of methods, including quantitative real-time RT-PCR, a method which incorporates a variety of means of detection, including hybridization probes." (See, Office Action, page 9.)

And the Examiner cites the following passage from the Bustin reference,

> "The accepted method for minimizing these errors and correcting for sample-to-sample variation is to amplify, simultaneously with the target, a cellular RNA that serves as an internal reference against which other RNA values can be normalized. The ideal internal standard should be expressed at a constant level among different tissues of an organism, at all stages of development, and should be unaffected by the experimental treatment. In addition, an exogenous control should also be expressed at roughly the same level as the RNA under study." (See, Office Action, page 10.)

The Backus reference does not teach the combination of a volume exclusion agent and a chemically-modified hot start DNA polymerase for the coamplification of two or more target nucleic acids wherein the nucleic acids are present at the beginning of the reaction in comparable copy numbers, i.e., the difference between the lowest and highest copy number of the nucleic acids to be amplified is not more than 10-fold. In fact, Backus, in reference to Sambrook et al., *Molecular Cloning, A Laboratory Manual*, page 9.50, (1989), teaches that, although volume exclusion agents are known to increase hybridization rates, they should only be used in PCR in a very limited set of circumstances, e.g., slow hybridization rates or where the nucleic acid is rate limiting in the reaction, because volume exclusion agents lead to high background and an increase in the viscosity of the reaction, making it difficult to handle. (See, Backus, column 2, lines 48-59.) In conclusion, Backus notes that, in light of the teaching of Sambrook et al., one skilled in the art would not use a volume exclusion agent in standard amplification methods as the hybridization rates are fast enough without its use and the target nucleic acids are not present in rate limiting amounts. (See, Backus, column 4, lines 47-51.)

However, according to Backus,

> "Moreover, one would expect that the [volume exclusion] agents would 'drive' all unfavorable reactions and create considerable unwanted product. This does not appear to be the case with the present invention." (See, Backus, column 4, lines 51-54.) (emphasis added.)

Backus only allows that the use of a volume exclusion agent in combination with an antibody-inactivated polymerase may be suitable under very limited hybridization conditions, e.g., where there is approximately **100,000-fold** difference in the levels of target nucleic acids, i.e., of high-copy number DNA and low-copy number DNA in the same reaction. Examples 1-4 of Backus disclose a PCR reaction with a DNA polymerase inactivated by the TP4 monoclonal antibody wherein the reaction is comprised of one population of low copy nucleic acid (10 copies of proviral HIV I DNA) and one population of high copy nucleic acid (1,000,000 copies of human β-globin DNA) amplified in the presence of 10% PEG. As seen in Table I of Backus, the best level of PCR amplification in the presence of 10% PEG 8000 only resulted in a 3.4-fold increase over control levels. In other words, Backus addresses the problems and limitations of standard PCR reactions when attempting to coamplify target nucleic acids that are present at the start of the amplification reaction in significantly disparate copy numbers to each other, for example adapting the concentration of primers to obtain comparable yields of the target nucleic acids.

In contrast, the present application demonstrates an improvement in a multiplex PCR reaction whereby multiple DNA targets present in comparable copy numbers can be co-amplified in high yield without resort to complicated manipulations of reaction time, hybridization conditions, or primer amounts. The key is the combined use of a thermostable hot start DNA polymerase and a volume exclusion agent. The data presented in Applicants' examples demonstrate that use of this combination leads to amplification of multiple targets instead of differential amplification, even where the copy numbers of the targets are comparable and even where the primers used are added in equimolar concentrations.

The Bustin reference is a review article describing methods for quantifying mRNA gene transcripts by reverse transcription polymerase chain reaction (RT-PCR). According to Bustin, RT-PCR-specific errors in the quantification of mRNA transcripts are compounded by variation in the amount of starting material, particularly when samples are taken from different individuals. The section of Bustin referred to by the Examiner concerns the normalization, i.e., calibration, to account for the errors that occur as a result of significant variations in the amount

of mRNA target transcripts in the starting material in RT-PCR reactions for quantifying mRNA transcripts again, particularly when comparing amplification of transcripts from samples taken from different individuals. According to Bustin, a common method for minimizing these quantification errors between samples is to include a cellular RNA in the reaction, one that is expressed at a constant level in various tissues and, if possible, expressed at the same level endogenously as the mRNA gene transcript under study. Therefore, Bustin relates to normalization of an RT-PCR reaction using an RNA with known *in vivo* expression levels.

It is submitted that Bustin provides a method for determining how badly a multiplex amplification has gone by including an internal standard as a target. No provision for improving the results of a multiplex amplification is provided, and no teaching that can be combined with the Backus reference suggests the improvement claimed by Applicants.

As discussed above, Applicants have demonstrated that their improved method is suitable for obtaining uniform amplifications in a multiplex PCR reaction, with multiple target nucleic acids, requiring multiple primer sets. Bustin does not disclose any means for leveling the output of a multiplex PCR.

In fact, Bustin indicates that, at the time the review was published (2000), Applicants' improved results were unknown. According to Bustin,

> "**Multiplex RT-PCR** . . .
> The final difficulty [with multiplex RT-PCR] is associated with limitations caused by mutual interference of multiple sets of PCR primers, which can reduce the dynamic range of the sensitivity and make quantification unreliable. Furthermore, the efficiency of multiplex detection is significantly affected by the extension time and the concentrations of dNTPs, primers and $MgCl_2$ . . . Vastly different levels of target mRNAs will cause quantification problems even for real-time detection, as the exponential phase of amplification of the less abundant mRNA will not overlap with that of the highly abundant target." (See, Bustin, page 185, right column, 3rd paragraph.)

Finally, Bustin concludes,

> "Therefore, in practice, if accurate quantification is the main aim, it is probably best to limit multiplexing to the detection of two or three transcripts." (See, Bustin, page 185, right column, 3rd paragraph.)

Thus, Bustin acknowledges the prevalence of the same problems in the art that are now addressed and overcome with Applicants' method. While the Bustin reference may identify

major problems existing in the field of multiplex RT-PCR, the best solution that is suggested for the problem by Bustin is reducing the number of target nucleic acids in the reaction mixture, a measure of caution not required when practicing the novel method disclosed in the present application. Bustin does not teach or suggest any aspect of Applicants' method for the coamplification of two or more target nucleic acids present in comparable copy number by employing a hot start DNA polymerase in combination with a volume exclusion agent, nor does Bustin suggest that such a reaction may be carried out without the need for primer optimization.

Birch et al. teach modification of a thermostable enzyme with a dicarboxylic acid anhydride and its use in a standard PCR reaction, i.e., to amplify a single target nucleic acid. However, Birch et al. do not teach or suggest the use of a hot start enzyme in combination with a volume exclusion agent for use in a multiplex PCR reaction. As such, the Birch et al. reference in combination with Backus and/or Bustin does not make up for the lack of this teaching or suggestion in either of those references.

In view of the foregoing remarks, the combination of Backus with Bustin and Birch et al. does not suggest the improvement of the present invention. Indeed, the improvement of the present invention overcomes problems that are acknowledged and illustrated by those publications. Accordingly, reconsideration and allowance of Claims 1, 2, 4-11, 16, 23, and 24 are respectfully solicited.

III. 35 U.S.C. §103

The Examiner has maintained the rejection of Claims 12-15 under 35 U.S.C. §103 as being unpatentable over Backus *supra*, in view of Bustin *supra*, and Birch et al. *supra*, and further in view of Reed et al., U.S. Pat. No. 5,459,038 ("Reed et al.") and Demeke et al., *Biotechniques*, 12(3): 333-334 (1992).

The Backus, Bustin, and Birch et al. references are as cited above. With respect to Reed et al. and Demeke et al., the Examiner states,

> "[I]t would have been prima facie obvious in view of the teachings of Demke [sic] and Reed to include dextran into the method of amplification taught by Backus in view of Bustin." (See, Office Action, page 14.)

The Demeke et al. reference simply reports that dextran did not inhibit PCR amplification of spinach DNA. (See, Demeke et al., "Results And Discussion")

And Reed et al. simply discloses a well known fact in the art of nucleic acid hybridization reactions, i.e., that inclusion of dextran in a PCR reaction increases the efficiency of amplification because, as is also disclosed in the present specification on page 6, lines 1-9,

> "It is known that the hybridization rate of nucleic acids is increased considerably in the presence of volume exclusion agents such as dextran sulfate or polyethylene glycol due to exclusion of nucleic acids from the volume of solution occupied by the agents."

Therefore, Demeke et al. and Reed et al., alone or in combination with Backus and/or Bustin, and/or Birch et al. do not teach or disclose Applicants' method of multiplex PCR amplification in the presence of a thermostable hot start DNA polymerase and a volume exclusion agent.

Reconsideration and allowance of Claims 12-15 are respectfully requested.

IV. 35 U.S.C. §103

The Examiner has maintained the rejection of Claim 25 under 35 U.S.C. §103 as being unpatentable over Backus *supra*, in view of Bustin *supra*, and Birch et al. *supra*, and further in view of Ivanov et al., U.S. Pat. No. 6,183,998 ("Ivanov et al.").

The Backus, Bustin, and Birch et al. references are as cited above. With respect to Ivanov et al. the Examiner states,

> "It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have extended the teaching of Bustin and Backus to include the chemical modification through reaction with an aldehyde as taught by Ivanov." (See, Office Action, page 15.)

Ivanov et al. show the use of a hot start specific DNA polymerase together with a non-ionic, polymeric volume exclusion agent. However, Ivanov et al. relates to PCR reactions in general and not specifically to multiplex PCR methods, and does not contemplate or disclose the particular improvement of the present invention which relates to a means for obtaining uniform multiplex PCR results while avoiding such techniques as optimizing the primer concentrations of all primers as necessary for multiplex PCR. A person skilled in the art would thus not take from Ivanov et al. any solution for the many problems associated with multiplex PCR.

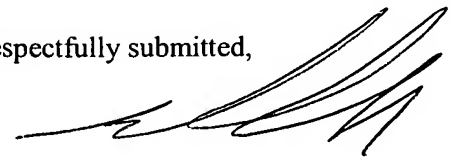
Accordingly, the combination of Ivanov et al. with Backus, Bustin or Birch et al. does not teach or suggest Applicants' method for multiplex PCR amplification.

Reconsideration and allowance of Claim 25 are respectfully requested.

For the reasons set forth above, none of the cited references, either alone or in combination, teach or suggest Applicants' improved method for the coamplification of two or more target nucleic acids.

Reconsideration and allowance of Claims 1, 2, 4-16, and 23-28, are respectfully requested.

Respectfully submitted,



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April 1, 2009
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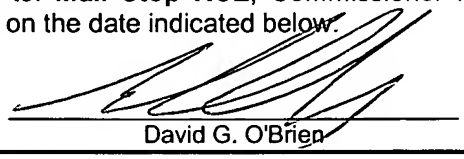

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